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BASIC INVESTIGATION

Screening of solanum surrattense for antibacterial, antifungal, phytotoxic and haemagglutination

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Abstract

OBJECTIVE: Plants produce a variety of useful bioactive materials that have been used to cure different ailments of human beings. With the same idea in mind, the crude methanolic extract and various fractions of Solanum surrattense were screened for antibacterial, antifungal, phytotoxic and heamagglutination activities.

METHODS: Standardized assays were followed for the determination of antibacterial, antifungal phytotoxic and heamagglutination activity.

RESULTS: The results of the antibacterial showed that crude methanolic extract was significantly active against Staphylococcus aureus (86%) The n-hexane fraction showed good activity against Pseudomonas aerogenosa (66.6%) and Bacillus subtilus (66.6%). The crude methanolic extract and various fractions were inactive against all test fungi. The crude methanolic extract, n-hexane, $CHCl_3$ and aqueous fractions showed moderate phytotoxic activities of 46.67%, 40.00%, 33.34% and 33.34% respectively at 1000 μg/mL. The crude methanolic extract and various fractions S. surrattense were unable to agglutinate RBCs of the human blood indicating that this species lack phytolectins.

CONCLUSION: The test sample showed significant antibacterial activity, no antifungal and heamagglutination activity while moderate phytotoxic was observed against Lemna minor L.

Key words: Solanum surrattense; Anti-bacterial agents; Antifungal agents; Phytotoxic; Haemagglutination

INTRODUCTION

Antibiotic resistance is one of the main problems faced by biological sciences the current era.[5-7] Among the available antibiotic and fungicides some possess serious side effects. Antibiotics like penicillin can cause allergy.[8-10] While other broad spectrum antibiotics kill the normal flora of the body thereby impairing normal body functions.[11-12] Resistance has also been seen among fungal species. Studies have shown the fungicides that are used against pathogenic fungal species; also have side effects.[7] Hence in order to develop new drugs that are safe and effective different strategies and methods have been followed. Scientists are trying their level best to find new and innovative antimicrobials agents that can cure diseases having no or fewer side effects.

One of the ways to find effective antimicrobial drugs is by searching nature itself.[13] Plants have been used to treat different infections for thousands of years.[14] Hence there has been a trend to screen different plants for their antibacterial and antifungal properties. The previous studies are convincing evidence that allelopathy has a potential role in natural and agricultural eco-

systems.[15] Extensive research has been conducted to find out the phenomenon involved in this mechanism for improving agricultural ecosystems. There has been a continuous effort to use natural products from plants as pesticides rather than synthesizing them in laboratories. Among different ways one is to screen plants for their allelopathic potential and to identify plants with phytotoxic capabilities.[15-17]

At present about 60% of the world's population uses medicinal plants to treat different health problems.[18] Plants are a rich source of bioactive compounds. Of the world 25 best selling pharmaceutical products, 12 are from plants.[19]

Solanum surrattense (Madaghoni) is a prickly herb with yellowish fruits. It is a common plant in North Africa, South & South East Asia, Australia and Polynesia.[20] It is also found in the dry mountainous regions of Pakistan and India.[20] S. surranttense has been used for many years to treat different diseases. The dried fruit powder is used as an internal medicine and the oil is extracted for the treatment of leucoderma.[20] It is also useful in cough, asthama, chronic rhinitis, dropsy, acute bronchitis and fever that are accompanied with chest infections.[20] The plant is also reported to possess anti-allergic activity.[20] Stem, flowers and fruits are bitter and carminative hence are prescribed for relief in burning sensation in the feet accompanied by vesicular watery eruptions.[21] Furthermore it is also believed that it can be useful in dengue fever, acute bronchitis and fevers accompanied by chest infections.[22]

Keeping in view the medicinal potential of S. surrattense, the current study was aimed to screen the crude methanolic extract and various fractions of fruits of S. surrattense for possible biological/pharmacological properties i.e. antibacterial, antifungal, phytotoxic and haemagglutination.

MATERIALS AND METHODS

Collection of plant material

The fruits of S. surrattense were collected from the dry hills of Lundkhwar, Mardan, Khyber Pukhtoonkhwa, Pakistan. The samples were kindly identified by Prof. Dr. Abdur-Rashid, plant taxonomist, Department of Botany, University of Peshawar, Pakistan.

Extraction

Fruits of the S. surrattense were kept in shade for drying. They were chopped into small pieces and grounded into powder using electric grinder. This fruit powder (640 g) was then soaked in methanol for 15 days (twice) at room temperature. Each time the mixture was filtered and then the filtrates were combined and concentrated with rotary evaporator at 40°C. A yellowish methanolic extract of 171 g was obtained.

Fractionation

The crude methanolic extract of S. Surrattense (150 g) was suspended in distilled water (350 mL). It was partitioned with n-hexane (3 × 500 mL), Chloroform ($CHCl_3$) (3 × 500 mL) and ethyl acetate (EtOAc) (3 × 500 mL) respectively, to yield the n- hexane (40 g), $CHCl_3$ (31 g), EtOAc (29 g) and aqueous (45 g) fractions. 20 g of the crude methanolic extract of was left for biological/pharmacological assays. All the fractions will only contain particular compounds based on the solubility of these compounds from the crude extract. For instance, the n-hexane fraction will contain only those compounds which are non-polar and so on.

Antibacterial activity

Antibacterial activity of the crude methanolic extract and various fractions of S. surrattense fruits were determined against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis. The method of Ahmad et al.[1] was undertaken used to find out the antibacterial activity. Eighteen hours old culture of the test organism from the nutrient broth was transferred to sterile nutrient agar plates to make bacterial lawn. After 15 min, wells were dug in plates using a sterile 6 mm borer. Stock solutions (3 mg/mL) of the test samples were prepared in sterile dimethyl sulfoxide (DMSO). 100 µl of crude methanolic extract and the fractions were loaded to their respective labeled wells. Amoxicillin and DMSO (less than 1%) were used as positive and negative controls, respectively. Zone of inhibition was measured (in mm) in comparison with positive control.

Antifungal activity

Antifungal activity of the crude methanolic extract and various fractions of S. surrattense fruits were determined against Aspergillus niger, Aspergillus flavus, Penicillium notatum, Fusarium oxysporum, Trichoderma harzianum and Alternaria alternata. For this activity the procedure of Bashir et al.[2] was employed. Test samples 24 mg/mL were dissolved in the sterile dimethyl sulfoxide (DMSO, Merck) to make stock solution. The SDA media was autoclaved and allowed to cool to about 55°C. After the temperature reaches to about 55°C, 67 µL from the stock solutions of the test samples was introduced to SDA media. Tubes were then allowed to solidify in the slanted position at room temperature. Each tube was inoculated with inoculums from a seven days old culture of test fungi. DMSO and standard antifungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed after 7 days of incubation at (28 ± 1)°C. Humidity (40%-50%) was controlled by placing an open pan of water in the incubator. The results were taken by measuring the linear growth of fungi in slants.

Phytotoxic activity

The phytotoxic activity of S. surrattense test samples (methanolic crude extract and fractions) was checked against Lemna minor L. available at the Department of

Botany, University of Peshawar. The method of McLaughlin et al[3] was followed for this activity. Stock solutions of the test samples were prepared in methanol at concentration of 20 mg/mL. E-medium was also prepared for the growth of L. minor. 10, 100 and 1000 µg/mL from the stock solution were introduced into three separate flasks and left at room temperature till methanol was evaporated. 20 mL of the E-medium and sixteen healthy plants with a rosette of three leaves were added to all the flasks and incubated at (28 ±1)°C for 7 days. Paraquat at a concentration 0.015 µg/mL was used as standard growth inhibitor. Results were taken by counting the number of damaged and healthy plantlets.

Haemagglutination activity

Crude methanolic extract and various fractions of S. surrattense were screened for possible haemagglutination activity, against human erythrocytes of all blood groups. The method of Naqvi et al[4] was followed. Fresh blood was collected from healthy volunteers, centrifuged at 6000 rpm for 10 min and the erythrocytes were separated. 2% erythrocytes suspension was prepared in phosphate buffer (pH 7.4). Stock solutions (1 mg/mL) of the test samples were prepared in DMSO and different dilutions (1 : 2, 1 : 4, 1 : 8 and 1 : 16) were made from it. From each dilution 1 mL was added to 1mL of 2% erythrocytes suspension and incubated at 37°C. Positive and negative results are indicated by rough granules and smooth button formation, whereas extent of deposition determined the intensity of positive result.

RESULTS AND DISCUSSIONS

Interest regarding research on medicinal plants has increased over the last two decades due to onset of new infection, e.g. infections by Enterococcus and Staphylococcus species. These are agents of many nosocomial infections and studies have indicated that they are resistance to available drugs. For example S. aureus has become resistant to several antibiotics to which it was previously susceptible. Some of the antibiotics to which S. aureus is now resistant are pencillin G, lincosamides, macrolides, tetracyclines, and gentamicin.[23]

Results for antibacterial activity of the crude methanolic and various fractions of S. surrattense are shown in Figure 1. The crude methanolic extract showed significant activity against S.aureus (86%), good against B. subtilus (77.7%) and moderate against P.aerogenosa (55.5%) and E.coli (44.4%). The n-hexane fraction showed significant activity against E.coli (81.5%), good against P.aerogenosa (66.6%) and B. subtilus (66.6%) and moderate activity against S.aureus (46.1%). $CHCl_3$ fraction showed good activity against S.aureus (65.3%), moderate against B. subtilus (37.0%), and P.aerogenosa (33.0%) and low against E.coli (14.8%). Moderate activity was shown by the EtOAc fraction against B. Subtilus (59.0%), S.aureus (57.6%), and E. coli (37.0%) and low against P.aerogenosa (14%). The aqueous fraction showed moderate activity against E. coli (40.7%), B. subtilus (40.0%), S.aureus (42.3%) and P.aerogenosa (33.0 %).

Our results for antifungal assay indicated that the crude methanolic and other fractions of S. surrattense were inactive against all the test fungi.

Lemna plants are miniature aquatic monocotyledonous plants that are very sensitive to bioactive compounds having phytotoxic properties. Lemna assay has been used to detect natural phytotoxic and anti-tumor compounds.[24] Hence it can be used to detect new plant growth inhibitors. Previously, we had also studied the phytotoxicity of the crude methanolic extracts of Ru-

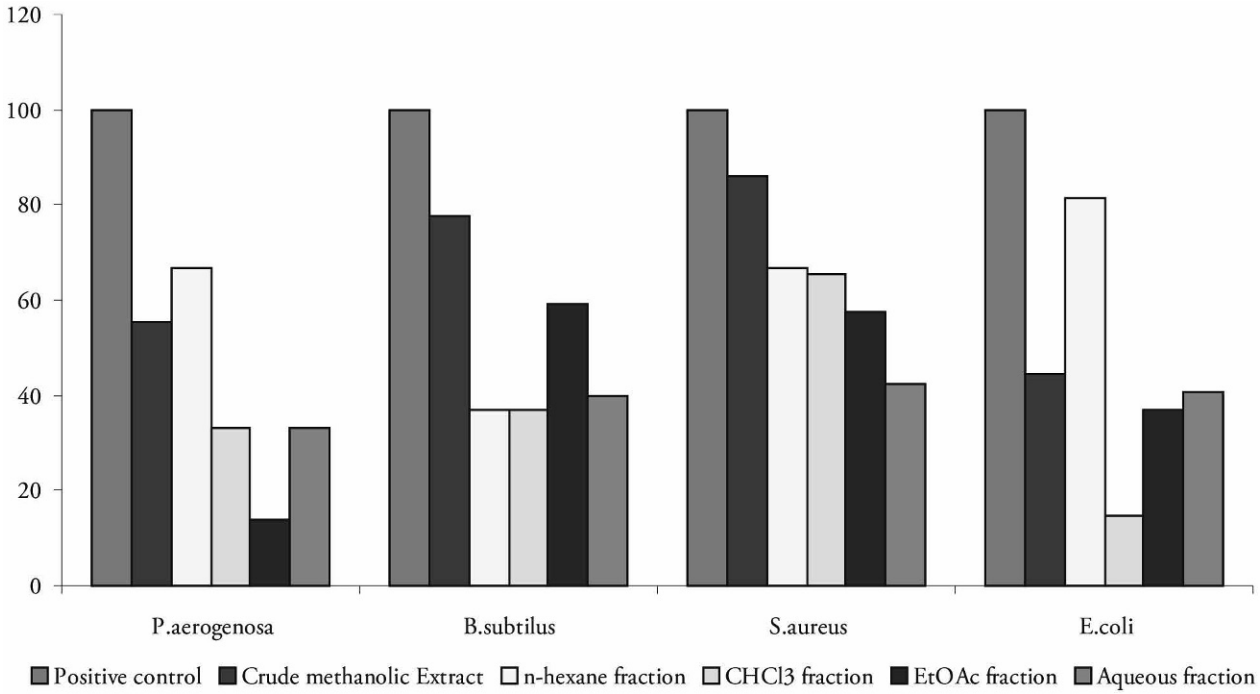

Figure 1 Antibacterial activity of crude methanolic extract and other fractions of S. Surrattense

mexhastatus, Rumex dentatus, Rumex nepalensis, Rheum australe, Polygonum persicaria and Polygonum plebejum (Family Polygonaceae) using the same Lemna bioassay.[25] The crude methanolic extract, n-hexane, $CHCl_3$ and aqueous fractions showed moderate activities of 46.67%, 40.00%, 33.34%, and 33.34% respectively at 1000 µg/mL. It can also be seen from the result that among all the fractions Ethyl acetate fraction had the least phytotoxic ability.

Lectins are found ubiquitously in plants and other organisms. Their ability to differentiate different carbohydrates moiety on cell surfaces (receptors) and in solutions, have given rise to speculations about their probable physiological role. Using this specificity of lectins, studies have been conducted on the structural and functional roles of cell surface carbohydrates.[26] It has also been used to identify sugar components of normal and cancerous cell and for agglutination of erythrocytes to find the blood type and for estimation of the number of virus particles.[27] Keeping in view this diverse role of lectins, haemagglutination activity of S. surrattense was done against red blood cells (RBCs) of human blood. However we found that all dilutions of the test samples (crude methanolic extract and various fractions) showed no haemagglutination activity against any blood group. The S. surrattense fractions were unable to agglutinate RBCs of the human blood which indicating that this species lack phytolectins.

We conclude that S. surrattense has a potential to be used as an antibacterial drug especially its crude methanolic extract against S. aureus and B. subtilis presents a good case to be investigated further into. From the results it can be also be suggested that S. surrattense do have significant phytotoxic activity and hence can be considered as an alternative to synthetic herbicides.

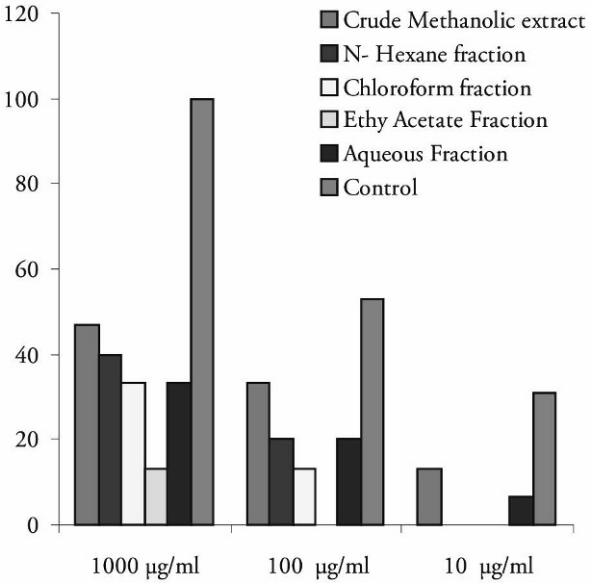

Figure 2 Phytotoxic activity of crude methanolic and other fractions of S. Surrattense

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